with the writer's compliments

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# ON THE CHROMATOLOGY OF SOME BRITISH SPONGES. By C. A. MAC MUNN, M.A., M.D. (Pl. I.)

In the *Journal of Anatomy and Physiology* for November, 1869[1], is an abstract by Professor Lankester of a report presented to the British Association at Exeter in 1869 "On the Spectroscopic Examination of certain Animal Substances" by himself. In that abstract he describes the colouring matter of *Spongilla fluviatilis*, which he calls chondrichlor, and which for certain reasons he considered not to be identical with leaf green. Sorby on examining it subsequently[2] found that it was chlorophyll, as it contained all the essential constituents of chlorophyll, which are soluble in carbon bisulphide, besides a yellow substance soluble in water, very similar to, if not identical with, one met with in many fungi. That some slight differences do exist between spongilla chlorophyll and plant chlorophyll is probable from the results of my own examination, published in the *Philosophical Transactions*, Part I. 1886, these differences being indicated by its behaviour on saponification.

In the abstract by Prof. Lankester above referred to he includes sea-water sponges among those animals whose pigments yield no bands.

Prof. Moseley in his paper "On the Colouring Matters of various Animals, and especially of Deep-Sea Forms dredged by H.M.S. *Challenger*[3]," describes a colouring matter from *Poliopogon Amadou* (Wyville Thomson) which was bright pinkish purple, soluble in dilute alcohol and fresh water, but gave no bands.

Krukenberg has made a great number of observations on sea-water sponges, and his observations have brought to light the existence of a number of colouring matters, such as lipochromes, floridines, uranidines, and hepatochromates.

He recognised the chlorophylloid nature of the last-named, but missed some of the bands. Otherwise doubtless he would have laid

[1] p. 119 et seq.
[2] *Quart. Journ. Micros. Soc.* Vol. xv. N. S. p. 47.
[3] *Quart. Journ. Micros. Soc.* Vol. xvii. N. S. p. 1—23.

more stress on their likeness to plant chlorophyll. It would occupy too much space to give Krukenberg's results in detail, so they will be referred to as briefly as possible. He has shortly summarized them in his "Grundzüge einer Vergleichenden Physiologie der Farbstoffe und der Farben" (1884).

In many sponges (*Suberitidae, Myxilla, Clathria* &c.) the bright colouration, orange, yellow or deep red, is due to the presence of yellow and rhodophan-like lipochromes. In no sea-water sponge examined by him were these entirely absent. In species of *Aplysina* a yellow uranidine (pigments to be referred to below) is present with the fat-colouring matter, and the black colouration which many of the *Caco-spongiæ* undergo and the colour change of the alcoholic extract of *Hircinia spinulosa* by saponification are due to the formation of melanotic decomposition products of the uranidines. In species of *Reniera*, floridines occur and mask the lipochromes, and the former also occur in *Hircinia variabilis.* These floridines show in their chemical behaviour many agreements with the pigments of red plant-leaves, red fruits, and those of red algae. But their spectra (as I have proved to my own satisfaction) are different.

It is necessary to describe these and the uranidines more in detail, referring however back to some of Krukenberg's earlier researches for fuller descriptions[1]. The floridines are violet to purple red in colour, soluble in water and glycerin, insoluble in media which dissolve the lipochromes. They occur not only in sponges but as haemerythrin in the blood of *Sipunculus nudus*, and in *Bugula neritina* (a Bryozoon). By deoxidation they are changed into chromogens, from which by the action of ferments and reception of oxygen they are capable of being regenerated. They are found in *Hircinia variabilis*, in certain species of *Spongelia* and *Reniera.*

In *Hircinia variabilis* one can easily press out a "superb" rose-coloured fluid from the canal-system which stains linen and paper of a rose colour, the stain lasting for a month. The aqueous extract shows a band between *D* and *E*; by the action of ammonia the colour changes to a dirty blue (green blue), and by that of hydrochloric acid to a beautiful purple, in both cases the colouring matter being precipitated. In the former case the single band is replaced by two, one before *D* and one between *D* and *E*, these however are finally replaced by one before and enclosing *D*. The watery solution loses its colour on heating to

[1] *Vergleichend-physiologische Studien.* Zweite Reihe, Dritte Abth. 1882, S. 22, &c.

from 60° to 70° C. The glycerin extract is rose-coloured, and has a green fluorescence in dilute, and yellow in concentrated solutions, and shows two bands, one between *D* and *E*, nearer *D*, the other between *b* and *F*, nearer *F*, the latter being continued into the general absorption of the violet end of the spectrum. In alcohol extracts of this sponge Krukenberg observed a band in red, which is doubtless due to the presence of a chlorophyll.

In *Reniera purpurea* from Trieste, a similar colouring matter was observed which withstood exposure to direct sunlight for many hours: its watery extract was decomposed by boiling. Ammonia precipitated the colouring matter out of an aqueous solution but without change of colour. The aqueous solution possessed a violet fluorescence, increased by acetic acid, but under the influence of this reagent the colour remained unchanged. Fuming nitric acid at once destroyed the colour, sulphuric and hydrochloric acids more gradually. The aqueous extract shows one broad band extending from about halfway between *D* and *E* to *F*, and becoming gradually merged into the general absorption of the violet end. These floridines are limited, according to Krukenberg's observations, to sponges which appear violet to purple red to the naked eye. It would be more satisfactory to have had other chemical and spectroscopic characters described more in detail, in order to enable one to judge whether one is justified in establishing a similarity to, or an identity with, Krukenberg's floridines in dealing with new sponge pigments.

The *Uranidines*, according to Krukenberg, are a class of pigments of a yellow colour which under certain influences, e.g. by ferments, are changed into brownish or dark-violet masses which are insoluble in lipochrome solvents, and refractory towards acids when thus changed. They are not limited to sponges, but occur also in the Ascidia and Insecta, where they form the "lymphatic colouring matters," (e.g. in *Hydrophilus, Dyticus, Oryctes, Melolontha,* the pupae of lepidoptera &c.); also as aethalioflavin in *Aethalium septicum* (a myxomycetous Thallophyte) and in *Holothuria poli.*

In *Aplysina aerophoba* a uranidine occurs which Krukenberg calls aplysinofulvin, and with it also four other pigments. Among the latter a body which is soluble in alcohol, ether, chloroform, benzol, and bisulphide of carbon, and which gives a band between *B* and *C*; this is evidently a chlorophyll and it was found not only in this sponge but also in *Reniera aquaeductus, Tedania muggiana, Suberites flavus, Clathria coralloides, Hircinia variabilis,* and *Tethya lyncureum.*

Krukenberg says its agreement with a widely-distributed hepatic colouring stuff among the invertebrates is in the highest degree probable. So that he recognised its chlorophylloid nature and its likeness to enterochlorophyll, but he does not give nearly all the bands. I shall have to refer again to this point. The second yellow pigment of the above sponge is one which also occurs in other sponges, e.g. *Tedania muggiana, Tethya lyncureum, Suberites flavus,* and *S. massa,* &c.; this was extracted from the saponified sponge extract by means of petroleum ether and ether. After the decomposition of the soap by hydrochloric acid in excess, ether removed again a yellow pigment, whose spectrum was free from bands, and this colouring matter was tolerably pure aplysinofulvin: the uranidine[1].

The second pigment just referred to shows two bands between *b* and *G*, and from Krukenberg's drawings and the data he furnishes[2] it appeared to be a lipochrome.

By direct extraction of the living tissues of *Aplysina* with alcohol, ether, or oil of turpentine often yellow-coloured solutions were obtained which showed a band before *E*, and the pigment soluble in these was insoluble in bisulphide of carbon, and therefore is a third colouring matter. Not seldom, too, bisulphide extracts showed a band before *D*, which belonged to a fourth pigment. The fifth pigment was the uranidine just referred to, "which in water, alcohol, ether, chloroform, benzol, petroleum-ether, oil of turpentine, &c. was likewise not insoluble," but in solution much more easily and more quickly decomposed by the oxygen of the air than when distributed in the dying tissues of *Aplysina.* This pigment preponderates over the other yellow pigments, and to it are to be attributed most of the reactions which are to be observed by direct treatment of an alcohol extract of *Aplysina* with various reagents. The alcohol extract, for example, is coloured by concentrated sulphuric acid at first yellow-brown, later red-brown; and by saponifying with caustic soda deep orange-red, occasionally also red-brown, and finally deep dark violet.

By pouring alcohol acidulated with hydrochloric acid on pieces of sponge, the tissues preserve their yellow colour for a day, but the affinity of the aplysinofulvin for oxygen outweighs the influence of the acid, so much so that after some time the pieces of sponge become

[1] Krukenberg's description is somewhat difficult to follow, and I have translated it as it stands in the original.

[2] Tafel III. sp. 4, 6, 7, 9, 11—14, *Vergl. physiol. Studien.* Zweite Reihe, Dritte Abth. 1882.

coloured at first red-brown, later deep, dark violet, and the acidulated alcohol extract assumes a brown-yellow colour, which cannot be removed by stronger acidulation. Krukenberg then goes on to show that the darkening of this pigment occurs under different circumstances to those which obtain in the darkening of the "lymph" of *Hydrophilus*, for in the latter case "melanosis" is prevented by heating to 54° C, whereas in *Aplysina* heating brings it about, as it at once blackens at a boiling temperature, which blackening requires in other cases a long exposure to oxygen. The acid solutions however are not thus changed by heat. *Aplysilla sulfurea* likewise contains a similar pigment: a uranidine. Krukenberg assumes the presence of an oxidation ferment in *Hydrophilus* lymph, whereas in *Aplysina* &c. he supposes the ferment to exert a reducing action, which inhibits[1] the production of the dark colour: this reduction ferment is destroyed by boiling; hence the darkening which then takes place. The solutions of aplysinofulvin are free from bands.

I may now briefly refer to Krukenberg's observations on the lipochromes of sponges before giving my own results. Sometimes, he says, we can easily recognise the bands of these lipochromes in solutions which are capable of dissolving them, sometimes however they are rendered invisible by the presence of other colouring matters. Saponification enables them to be prepared in a purer state, a process which however decomposes the chlorophylloid pigments, which Krukenberg calls the "hepatochromatic colouring matters," but he found that bands of the lipochrome in unsaponified extracts corresponded with bands of similar solutions from saponified extracts. A lipochrome which in the solid state coloured itself deep blue with nitric and sulphuric acid, but remained unchanged with iodine, which was soluble in the usual lipochrome solvents and appeared to be identical with lipochrin, was found in *Tedania muggiana*. In petroleum-ether it gave one band covering *F*, and another placed between *F* and *G* (after saponifying). The ether solutions showed however, after the petroleum-ether had extracted all colouring matter soluble in it, only one band like that of Kühne's rhodophan, but differing from its band. Another pigment which occurred in red flocks and was left undissolved by the ether was insoluble in alcohol, chloroform, and bisulphide of carbon, but dissolved in acetic ether and dilute alkalies, and by "damping" with acetic acid became a deep purple, and then dissolved in bisulphide with a purple

[1] I use the word "inhibit" here, as this expresses the sense of Krukenberg's statements more tersely than an exact translation.

violet colour; these reactions agree with those of rhodophan (Krukenberg).

From the yellow alcohol extract of *Suberites massa* petroleum-ether extracted a like pigment with similar bands, and accompanying it was a red pigment which could not be separated from the yellow.

In *Suberites flavus*, by saponifying, purer yellow pigments were met with, and in such quantity that repeated extraction with petroleum-ether was required in order to separate from the soap all the colouring matter. This lipochrome is evidently identical with the last.

In another *Suberites* species another lipochrome was met with, not identical however with the last two. The band in this case resembles that of rhodophan in being single, but differs in position.

In *Papillina suberea* alcohol extracts another lipochrome, which after saponifying and extracting with petroleum-ether and evaporating to dryness is coloured blue by strong nitric and sulphuric acids and a distinct greenish by iodine (dissolved by means of iodide of potassium).

These then are the most important facts which Krukenberg has described: he refers to the lipochromes of other sponges, for which I must refer to his paper "Zur Kenntniss der Verbreitung der Lipochrome im Thierreiche" in "*Vergleichend-physiologische Studien*," Zweite Reihe, dritte Abtheilung 1882 (s. 92 et seq.).

I may here remark that an inspection of the plates accompanying Krukenberg's papers allows one to conclude the presence of a chlorophylloid pigment in the following sponges:—

1. *Hircinia variabilis.*
2. *Aplysina aerophoba.*
3. *Tedania muggiana.*
4. *Reniera aquaeductus.*
5. *Suberites flavus.*
6. *Tethya lyncureum.*
7. *Clathria coralloides.*

So far as I know I have given all the references to sponge chromatology, and I shall now give the results of my own examination. The species were identified for me by C. Jeffreys, "Naturalist," Tenby, who has sent the specimens for examination. Tenby is remarkable for the great number of sponges which it furnishes, as anyone knows who has explored its numerous caves. The nomenclature adopted here is that of P. H. Gosse, F.R.S.

*Halichondria panicea.* The specimens examined were greenish, and after extraction with absolute alcohol for a week in the dark yielded a

solution which was a dull green colour, with a distinct red fluorescence, and showed four of the usual chlorophyll bands, and at least one lipochrome band. (See Plate I. sp. 1.) These read as follows:—

1st. from shading to shading, including edges,
$\lambda$ 688·5 to $\lambda$ 644; darker part from
$\lambda$ 681·5 to $\lambda$ 656.
2nd. from $\lambda$ 619 to $\lambda$ 597.
3rd. a doubtful shading.
4th. from $\lambda$ 542 to $\lambda$ 529.
5th. approximately from $\lambda$ 492 to $\lambda$ 471.

On applying the fractional method to this solution (which was first applied by Professor Stokes and later by Dr Sorby[1] to solutions of chlorophyll), i.e. by agitating the solution diluted with water with bisulphide of carbon, the bisulphide layer was seen to be yellow, not green[2]. I attach great importance to this result because the same thing happens in solutions of enterochlorophyll. The bisulphide layer gave however the four chlorophyll bands, had a faint red fluorescence and strongly absorbed the violet end of the spectrum, sp. 2: while in a thinner layer at least two bands were visible still nearer the violet end, sp. 3.

The following are the readings of all the bands:—

1st. from $\lambda$ 695·5 to $\lambda$ 665 = dark part, shaded to $\lambda$ 647.
2nd. from $\lambda$ 630 to $\lambda$ 605.
3rd. a shading only from $\lambda$ 589 to $\lambda$ 569 (?)
4th. from $\lambda$ 550·5 to $\lambda$ 536·5.
5th. from $\lambda$ 523 to $\lambda$ 499 (?)
6th. from $\lambda$ 492 to $\lambda$ 473 (?)

The alcoholic solution from which this bisulphide layer had been separated was amber yellow in colour, seemed to have a faintly red fluorescence, and showed a faint chlorophyll band in red, and another at the blue end of green. It was again agitated with bisulphide of carbon, which formed a yellow layer, this again showed the bands of the same constituents as before. After the removal of the bisulphide the alcohol was still yellow, showed a feeble band in red, and some shading at the blue end of green. On making a second extract by means of

[1] I regret that I have not been able to carry out this fractional separation as completely as it can be carried out in the case of plant chlorophyll, owing to dearth of material. It is for the same reason that I have given the experiments in detail and have avoided general assertions.

[2] In the case of plant chlorophyll the bisulphide layer is green.

absolute alcohol of the same sponge a similar solution to the above was obtained. On evaporation, such a solution left a dirty brownish-green residue, which was partially soluble in rectified spirit, and what was left untouched was soluble in bisulphide of carbon, forming an orange coloured solution, showing however the same bands as the above bisulphide extracts. On evaporating this, the residue became green with nitric acid, dirty blue and green with sulphuric acid, and remained unchanged with iodine in iodide of potassium.

The portion of this residue soluble in rectified spirit formed a yellowish-green solution with a red fluorescence, giving the same bands as the original alcohol extract. On adding to it a little nitric acid a precipitate formed and the colour became slightly greener, which change was better marked with more nitric acid, while the fluorescence disappeared, and now its bands read :—

1st. from $\lambda$ 671 to $\lambda$ 641.
2nd. from $\lambda$ 612·5 to $\lambda$ 591.
3rd. from $\lambda$ 577 to $\lambda$ 555.
4th. from $\lambda$ 542 to $\lambda$ 523.

This result showed that we are here dealing with a chlorophyll beyond doubt. One does not always get such satisfactory results as the above, for some specimens appear to contain more chlorophyll than others. Thus in the filtered absolute alcohol extract of another sponge which had a yellowish-green colour and a fine red fluorescence the only well-marked band was that in red, still even in this case I could determine the presence of the same constituents as before.

By examining portions of the living sponge with the microspectroscope I failed to determine the presence of a band in red, but the absorption of the violet was well marked. On squeezing out a portion, a band occupying the blue half of the green may be seen. The outer surface of the sponge is much greener in a section than the interior, which is dull green, and here one can see a faint narrow band in the green of the spectrum, a band which becomes more distinct by treatment with ammonium sulphide; this probably belongs to a histohaematin, and can be seen much better marked and generally accompanied by another in glycerin extracts, but the extraction by means of glycerin should be carried out for at least 13 days. Such an extract is greenish, and a deep layer examined by gas-light has a reddish tint, and gives sp. 4. The first band here reads from $\lambda$ 566 to 559 and is intensified by sulphide of ammonium. This pigment also occurs in other sponges, as

will be shown. Hence *H. panicea* contains at least three pigments, (1) a chlorophyll, (2) a lipochrome, (3) a histohaematin.

*Halichondria caruncula.* The specimens examined had a dull orange-red colour. Pieces of sponge itself when examined in sufficiently thin layers transmit red, yellow and a little green, strongly absorbing the violet end of the spectrum; thin enough portions may show a dark shading in the green, in other parts one nearer the blue may be seen.

The alcohol extract was of a fine orange yellow colour with a decided red fluorescence, it showed a band in red and another before *D* (sp. 5), and strongly absorbed the violet end. In a thin layer a band became detached at blue end of green. The former bands read as follows :—

1st. from $\lambda$ 678 to $\lambda$ 653.
2nd. from $\lambda$ 625 to $\lambda$ 597.

The shading at the red end looked to me as if a second band nearer the red than the dominant chlorophyll band was present, and this supposition proved to be correct on adopting the fractional method of separation, as it became plainly visible in the bisulphide of carbon. The lipochrome constituent was masked by the pigment which had such a strongly absorbent power for the violet end, so that its bands could not be measured, but there appeared to be a shading in an absolute alcohol solution from $\lambda$ 492 to $\lambda$ 471. On agitating a diluted alcohol solution with carbon bisulphide the latter sank down of an orange colour, leaving the spirit pale yellow, and showed a band in red which was really made up of two placed closely together. The violet end of spectrum being strongly absorbed.

The first band in red extended from $\lambda$ 712 to $\lambda$ 699.
The second from $\lambda$ 685 to $\lambda$ 650.

I have never noticed a band so far over in red before, and its occurrence stamps this as a purely animal pigment. In a thin layer of the bisulphide extract there were two lipochrome bands: the first from $\lambda$ 520 to $\lambda$ 496, and the second from about $\lambda$ 490 to $\lambda$ 469. The spirit solution from which the bisulphide layer had been removed gave a faint band in red and some shading between green and blue, but no distinct lipochrome band could be seen in it.

On evaporating a bisulphide extract, obtained as just described, it left a residue of a yellow-brown colour, which became a dirty greyish green with iodine in iodide of potassium, with nitric acid a transient blue-green, blue and greenish passing into dirty grey, with strong sulphuric acid a dark dirty blue-green and green passing into brown.

On evaporating down an alcohol extract of this sponge a brown greasy looking residue is left, which now is not quite soluble in absolute alcohol, the latter becoming coloured yellow, having a greenish tint and a red fluorescence, absorbing strongly the violet end of the spectrum and showing in thin layers a band at blue end of green; and in a suitable depth the double band in red which is shown in sp. 6. The bands of this spectrum read:—

1st. $\lambda$ 712 to $\lambda$ 692.
2nd. $\lambda$ 678 to $\lambda$ 647; (dark part = $\lambda$ 674 – 5 to $\lambda$ 659).
3rd. $\lambda$ 619 to $\lambda$ 597.

On extracting the residue, left untouched by alcohol and bisulphide, with water, it dissolved, forming a yellow solution, and in this there was a trace of a band in red and one at blue end of green. An aqueous extract of the sponge itself was faintly blueish and showed some shading from about $\lambda$ 504 to $\lambda$ 481, which shading was not removed by hydrochloric acid nor caustic soda, although with the latter the colour became faint yellow. Hence in *Halichondria caruncula* a chlorophyll and a lipochrome beside other less well-defined pigments are present.

*Halichondria rosea.* The specimens examined were a kind of greyish colour with a rosy tint. No distinct bands could be seen in portions of the sponge. On placing some portions in water, after a short time the solution assumed a kind of rose pink colour, and this gave two bands, sp. 7, which measured:—

1st. $\lambda$ 511 to $\lambda$ 496.
2nd. $\lambda$ 490 to $\lambda$ 471 (?).

No remarkable change took place in this solution with ammonium sulphide; with Stokes's fluid the bands seemed better marked, but they could not be made to disappear by agitation with air. Caustic soda intensified them, and the solution became less rosy. Acetic acid did not affect either colour or spectrum. On boiling and adding a little acetic acid a faint opalescence was noticed. If this is a floridine it does not agree in spectrum with those given by Krukenberg.

In the glycerin extract of another specimen I detected the presence of a histohaematin, with one band at least from $\lambda$ 566 to $\lambda$ 559, this band being intensified by sulphide of ammonium, which also brought out another nearer violet too faint to be measured.

The rectified spirit extract was yellow and showed a faint red fluorescence. A deep layer gave sp. 8, and a shallow sp. 9. These bands read as follows:—

1st. λ 681·5 to λ 653.
2nd. λ 619 to λ 597.
3rd. doubtful.
4th. λ 545 to λ 532. (?)
5th. λ 499 to λ 473.

The solution was then agitated with bisulphide of carbon; the latter became coloured deep yellow, leaving the spirit almost colourless, and showed the band in red, a second faint band and the fourth, while the violet end was strongly absorbed. The following are the approximate measurements of these bands:—

1st. λ 688·5 to λ 671, = dark part; and shading to λ 665.
4th. λ 550·5 to λ 538.
5th. λ 520 to λ 496.
6th. about λ 490 to λ 469. (?)

By daylight however there was only one broad band, probably made up of two, visible, sp. 10. The united bands reading from λ 519 to λ 471. On evaporating the bisulphide down it left a brown-yellow residue which was quite yellow in a thin layer. With iodine in iodide of potassium it became perhaps slightly redder, with nitric acid deep green and blue-green, with sulphuric acid deep green, blue-green and finally dirty brown. The rectified spirit after separating this bisulphide extract showed a faint chlorophyll band in red, but no distinct lipochrome bands. On being concentrated by evaporation it showed however all the four chlorophyll bands and had a red fluorescence.

Hence *Halichondria rosea* contains chlorophyll, a lipochrome and another rose-coloured pigment soluble in water, which appears to be somewhat like a floridine, besides a histohaematin.

*Halina Bucklandi.* This is a black incrusting sponge; a thin section examined by gas-light seemed brownish.

The absolute alcohol extract was greenish, and it had a red fluorescence, the spectrum of which is shown in sp. 11 and sp. 12; these bands read as follows:—

1st. λ 681·5 to λ 647; dark part = λ 678 to λ 659.
2nd. λ 625 to λ 597.
3rd. about λ 591 to λ 573.
4th. λ 545 to λ 532. (?)
5th. λ 492 to λ 469.

So that *Halina Bucklandi* contains a chlorophyll[1] and a lipochrome. I could not further examine this solution owing to dearth of material.

[1] A faint histohaematin band was present in the sponge itself.

*Halichondria incrustans.* Dull yellow specimens were examined, with here and there greenish parts on the surface which were parts of the sponge itself, not due to algae growing upon it.

On examining a portion of the sponge itself with the microspectroscope it was found to cut off the violet end of spectrum to about half of green, the red was transmitted apparently free from any band. In the green a faint narrow band was seen, sp. 13. The pigment giving this band is soluble in glycerin, which solution after 17 days' extraction was of a yellow colour and gave sp. 14; the bands of which were intensified by ammonium sulphide. The alcohol extract was yellow with a red fluorescence, and showed in a deep layer the chlorophyll bands, the dominant band of which here read,—λ 678 to λ 656. In a layer 98 millimetres deep I could not measure the other bands, hence the amount of chlorophyll in this sponge is relatively small. In this solution two lipochrome bands were seen:—

1st. λ 499 to λ 475.
2nd. λ 464 to λ 446.

On agitating with bisulphide of carbon, the latter fell down of a yellow colour, showing one band in red and two lipochrome bands placed closely together. The first about λ 529 to λ 499, and the second from λ 492 to λ 471. On evaporating this down it left a yellow residue, which remained unchanged with iodine in iodide of potassium, became with nitric acid a transient blue, blue-green and blue, with sulphuric acid a dirty slate-blue passing into brownish-violet. The alcohol solution from which the bisulphide had removed the above constituents still contained some chlorophyll and a lipochrome, and left on evaporation a residue of an orange-yellow colour; on adding absolute alcohol the whole residue was not taken up; the alcohol was yellow and showed all the usual series of chlorophyll bands and two lipochrome bands which (approximately) read:—

1st. λ 496 to λ 473.
2nd. λ 464 to λ 446.

Hence *Halichondria incrustans* contains chlorophyll and a lipochrome, as well as a histohaematin.

*Halichondria seriata.* The specimens examined were brick-red in colour, and this colour was uniformly distributed throughout the sponge-substance. A solid piece transmitted red and a slice of green, while in a thiner layer there was a distinct dark band at the red end of green, reminding of the tetronerythrin of *Homarus vulgaris* when in the solid state. A red juice exuded on squeezing the sponge, which

on adding water and filtering left the colouring matter on the filter paper, while the filtrate was yellowish and gave a shading in the blue-green, but no marked effect was produced by ammonium sulphide. In the glycerin extract after 17 days' extraction a decided red tint was apparent, and this showed a band at blue end of green but no histohaematin band, still with ammonium sulphide a very faint shading, in its position, appeared.

The absolute alcohol extract was reddish-yellow in deep layers but yellow in thin layers. It absorbed the violet end of spectrum up to about $E$ in a suitable depth, and showed one chlorophyll band in the red. In a very thin layer a band was seen at blue end of green, and another in violet; the former from about $\lambda$ 492 to $\lambda$ 471, and the latter from $\lambda$ 462 to $\lambda$ 444. (?)

On evaporation this solution left an orange-yellow residue, which gave with nitric acid a transient blue and greenish colour, with sulphuric acid a slate blue, and remained unchanged with iodine in iodide of potassium. On adding water and agitating with bisulphide of carbon, the latter settled down as an orange solution and did not show the chlorophyll band in the red, but strongly absorbed the violet end of the spectrum up to beginning of green in a moderately deep layer, while in a thinner layer two distinct bands became detached: the first from $\lambda$ 520 to $\lambda$ 494, the second about $\lambda$ 490 to $\lambda$ 469.

The alcohol solution from which this was removed was orange in colour in deep layers but yellow in thin layers, and showed a feeble band in red, and the lipochrome bands slightly changed in position owing to admixture with some bisulphide.

It was now necessary to find out whether by concentration of an alcohol solution the other chlorophyll bands could be brought into view, and, on evaporating, an orange residue with some brown particles was left; on extracting these with absolute alcohol some brownish greasy masses were left undissolved, the alcohol assuming a fine orange-red colour (gas-light), and now the second chlorophyll band was plainly seen, also at least one lipochrome band from about $\lambda$ 490 to $\lambda$ 469. On extracting the portion of residue left untouched by the alcohol with ether the latter assumed an orange colour, and a little residue was left which went completely into water, and the solution showed a band at blue end of green, and absorbed the violet end of spectrum. In the aqueous extract aggregate of prismatic crystals formed which closely resembled creatin. But on applying the tests for creatin I obtained a negative result, nor did I obtain any evidence of the presence of uric acid. They

effervesced with nitric acid without any change of colour. Crystals of chloride of sodium were met with often in such extracts, but these did not belong to chloride of sodium, and their nature at present remains doubtful. So far we have present in *Halichondria seriata* chlorophyll and at least one lipochrome, the presence of a histohaematin is doubtful.

*Hymeniacidon albescens.* The specimens examined were of a yellow colour, and a distinct histohaematin band was visible in sections, with good illumination; and in thin enough parts a band at the blue end of green. On steeping for six days in absolute alcohol and filtering a pale yellow solution was obtained which did not show a chlorophyll band, but did show two lipochrome bands: the first from about $\lambda$ 499 to $\lambda$ 475, and the second very doubtful from about $\lambda$ 464 to $\lambda$ 446. On evaporating and dissolving in carbon bisulphide a much deeper yellow solution was obtained, which strongly absorbed the violet end of spectrum up to beginning of green and showed no chlorophyll band, while the lipochrome bands now read:—

1st. $\lambda$ 526 to $\lambda$ 499.
2nd. $\lambda$ 492 to $\lambda$ 471.

On evaporation this left a gamboge and orange coloured residue, which remained unchanged with iodine in iodide of potassium, became dirty green and transient blue with nitric acid, and a kind of dirty greyish-green and in parts deep blue with sulphuric acid, passing into brown or violet brown.

Hence *Hymeniacidon albescens* contains no chlorophyll, but does contain a lipochrome and a histohaematin.

*Halichondria albescens.* A yellowish sponge. Sections showed a distinct band at blue end of green and a decided though faint histohaematin band. The glycerin extract was pale yellow, and showed a distinct histohaematin band and a faint second one. The former read about $\lambda$ 566 to $\lambda$ 559, intensified by sulphide of ammonium. The alcohol extract was pale yellow, showed no chlorophyll band, but another at blue end of green about $\lambda$ 499 to $\lambda$ 475, and possibly another nearer violet. The solution on evaporation left a yellow and in parts a deeper yellow residue. On solution in absolute alcohol the latter became yellow in colour and showed no band in red and the above-mentioned one. The residue remained unchanged with iodine in iodide of potassium, became a dirty green or greyish with nitric acid, and a brilliant red with sulphuric acid. Hence *Halichondria albescens* contains no chlorophyll, but does contain a histohaematin and a body like a lipochrome, but yet different from one.

In another specimen of the same species I failed to get the red reaction with sulphuric acid (from the residue left after evaporation of an alcohol solution); and with nitric acid the result was equally negative. The residue was soluble entirely in water, however, with a yellow colour but gave in this no noticeable band. Possibly it had become changed by heat.

*Grantia coriacea.* A dull orange-red sponge. Portions absorbed the violet end of spectrum strongly up to the beginning of the green, and a thicker part only transmitted the red rays. In very thin parts some shading in green was perceptible.

The glycerin extract was brown in colour, reddish brown by transmitted gas-light, and let through only the red of the spectrum in deep layers, in shallower some shading at the end of green, and showed no histohaematin band before or after adding ammonium sulphide.

The absolute alcohol extract was orange in deep layers, yellow in thin, absorbed the violet end of spectrum strongly, transmitting red and half of green. In thinner layers a band was seen at the end of green, and a doubtful second in violet: the first from $\lambda$ 501 to $\lambda$ 475, second from $\lambda$ 464 to $\lambda$ 446.

This solution when heated on the water-bath underwent a change of colour from yellow to a dirty dark green, finally almost to black green; although green with reflected light, yet with transmitted it was a kind of brown-violet, and this solution gave a band before $D$, somewhat like that of sp. 15, while as is there shown the violet was strongly absorbed. When to this solution I added sulphide of ammonium the band disappeared, the colour changing to brownish. Ammonia brought about the same colour change but the band persisted; when after the ammonia ammonium-sulphide was added the band did not disappear. The band of a diluted solution read approximately from $\lambda$ 641 to $\lambda$ 597. Hydrochloric acid changed the colour to brownish yellow and no bands were seen. Nitric acid produced the same effect. The residue left on complete evaporation of the above alcohol solution was dark brown and amorphous, in thin layers it was greenish. Absolute alcohol left it undissolved in greater part, taking up some pigment which coloured the alcohol yellow; this extraction was repeated as long as the alcohol took up anything. The resulting solution was a deep yellow colour and strongly absorbed the violet end of the spectrum in deep layers, while in shallow a band became detached at blue end of green from about $\lambda$ 496 to $\lambda$ 475, and another from about $\lambda$ 464 to $\lambda$ 446. On evaporating the solution, the residue was a canary-yellow

colour, in parts of an orange or orange-red. Bisulphide of carbon dissolved this residue, forming an orange solution showing two bands placed closely together: the first from about λ 529 to λ 502, and second λ 492 to λ 471. The residue left by evaporation of such a solution remained unchanged under the influence of iodine in iodide of potassium, turned blue and then green, but transiently, with nitric acid, and a fine dark green and bluish-green with sulphuric acid. Hence this pigment taken up by the alcohol was a lipochrome, and it is quite evident that this was not the pigment which turned dark green by heat.

The residue left after the alcohol had removed this lipochrome was a mixture of dirty brown and green, from which chloroform and bisulphide of carbon still removed some colouring matter; but the dirty green and brown residue was still insoluble in these and went into water, forming a brown solution, also in part into glycerin. The former solution was brown with transmitted gas-light, and deep red in a deep layer. In the latter depth red was alone transmitted spectroscopically, while in a shallow layer a band like that of sp. 15 was seen. Neither by means of hydrochloric acid nor caustic potash could the colour be made to disappear, but the band became indistinct under the influence of the latter reagent.

Hence in *Grantia coriacea* a chromogen was present which by boiling became of a dark green colour, and this chromogen is evidently related to Krukenberg's Aplysinofulvin. But whether the boiling destroyed a ferment which previously inhibited the change of colour from yellow to dark green or whether the heat changed the molecular condition of the original yellow pigment could not be decided by the result of such experiments. A comparison of this result with those of Krukenberg allows one however to infer that a uranidine was here present, and one may also safely conclude that this uranidine was quite distinct from, and independent of, the lipochrome which accompanied it.

In another specimen which seemed to belong to the same species some further experiments were made which may be here added. The glycerin extract was brown, it showed no well-marked band, but in deep layers absorbed the violet end of spectrum, strongly transmitting red and a little green, in a thinner layer a shading at blue end of green was noticed. No histohaematin could be detected, either with or without the use of sulphide of ammonium. Ammonia left the colour unchanged; acetic acid made it lighter, but developed no bands. Hydrochloric acid changed the colour to yellow (faint), but no bands were noticed.

An absolute alcohol extract was of an orange colour in a suitable

depth, showed a faint chlorophyll band in red, and strongly absorbed the violet end to the middle of green. In a thin layer there was a dark band at blue end of green, and possibly another in the violet which read :—1st. λ 499 to λ 475, and 2nd. λ 464 to λ 446. On evaporating over the water-bath the colour became—after an initial yellow and green stage—a dark brown violet. The residue appeared dark brown, in other parts brown-green, and in very thin layers a dirty green. On extraction with absolute alcohol this solvent assumed an orange-yellow colour, and after its action the residue showed a peculiar appearance, being arranged in concentric circles of deposit arranged in brown and green circles. The absolute alcohol solution strongly absorbed the violet end and showed a faint band in red; while in sufficiently thin layers two lipochrome bands were seen; the first from λ 496 to λ 475, the second from λ 462 to λ 444. This left on evaporation by means of heat an orange residue which was soluble in bisulphide of carbon, forming an orange solution strongly absorbing the violet end of spectrum up to beginning of green, while in less deep layers two bands were seen: the first from λ 529 to λ 502, the second λ 492 to λ 471. The residue was also soluble in ether, showing similar bands, the first from λ 499 to λ 475, the second from λ 464 to λ 446. The residue from this showed hardly any change with iodine in iodide of potassium (perhaps it was slightly more green), with nitric acid an evanescent blue and greenish, with sulphuric acid a fine blue, and green passing into a kind of violet-red or violet-brown. This lipochrome was also soluble in turpentine, forming a deep yellow solution showing two bands: 1st. λ 502 to λ 479, and 2nd. λ 467 to λ 450.

After the extraction of the lipochrome from the residue, the latter assumed after the lapse of some time a dark blue-green colour, and although insoluble in the lipochrome solvents it went at once into water, which viewed by gas-light in a white dish was a deep claret colour in the deep parts but greenish at the edges, but with transmitted gas-light it was brownish, and in some lights deep red. In deep layers it transmitted the red rays only, while in thinner layers a band before *D* (such as that shown in sp. 15) was visible. The darkest part of this band read from λ 630 to λ 593, and the shading of its edges from λ 647 to λ 562. With ammonium sulphide this band disappeared, the colour changing to light yellowish brown. With ammonia it became dark brown and the band persisted. With caustic potash it turned decidedly greenish, and the band of sp. 15 now stood at from λ 665 to λ 630 (= darkest part). With acetic acid it became yellow-brown and only a

trace of the band was left. With hydrochloric acid it became reddish-yellow and the band disappeared. Nitric acid changed it to red-yellow and no band was seen: sulphuric acid produced the same effect.

Hence in this second specimen of *Grantia coriacea* a uranidine and a lipochrome with a trace of chlorophyll were present. But both the latter were independent of, and had apparently nothing to do with, the development of the uranidine.

*Halichondria sanguinea.* A scarlet sponge. A portion of the sponge itself transmitted only a slice of red, while in a thin layer a broad and ill-defined band was seen at the beginning of green. The rectified spirit extract was of a golden-yellow colour with a fine red fluorescence, and gave in deep layers sp. 16, and in shallow sp. 17.

Only one of these bands could be certainly measured, and it read from $\lambda$ 678 to $\lambda$ 653, and its dark part from $\lambda$ 674·5 to $\lambda$ 659. The lipochrome band read approximately from $\lambda$ 502 to $\lambda$ 471. (?) This solution was agitated with bisulphide of carbon, which sank down of a deep orange colour, showing a band in red, and in less depths the traces of three bands, nearer violet, evidently those of a lipochrome.

The rectified spirit extract from which the bisulphide had removed the above was pale yellow, and showed a band in red and one lipochrome band.

On evaporation, the bisulphide extract left some brownish residue, which in thinner parts was yellow; this gave with iodine in iodide of potassium a distinct green colouration, with nitric acid a fine but transient blue passing into greenish, with sulphuric acid a bluish-green, green and blue passing into brown. It was now necessary to see whether the whole series of chlorophyll bands were present in a concentrated alcohol solution: accordingly the residue left after evaporation of a bisulphide extract was dissolved in absolute alcohol, when it formed a deep yellow solution with a red fluorescence and giving the following bands:—

1st. from $\lambda$ 678 to $\lambda$ 650.
2nd. $\lambda$ 625 to $\lambda$ 597. (?)
3rd. $\lambda$ 591 to $\lambda$ 573.

After a bisulphide solution had been removed from a rectified spirit extract and the latter evaporated down and dissolved in bisulphide it showed two lipochrome bands, which read:— (approximately)

1st. $\lambda$ 520 to $\lambda$ 494.
2nd. $\lambda$ 485 to $\lambda$ 466.

And the residue left after evaporation of this solution became slightly

green with iodine in iodide of potassium, a transient blue and greenish with nitric acid, and greenish with sulphuric acid.

Hence *Halichondria sanguinea* contains chlorophyll and one or two lipochromes.

*Leuconia Gossei.* A yellowish-white sponge. A section absorbed some of the violet, and showed a band at blue end of green, also a faint histohaematin band.

The glycerin extract was pale yellow, and slightly reddish in deep layers by gas-light, and with ammonium sulphide showed a faint histohaematin band.

A rectified spirit extract was pale golden yellow, and showed a feeble chlorophyll band in red, and a shading between green and blue. It was concentrated by heat and left a dirty brown-yellow residue, only partially soluble in absolute alcohol, forming a pale yellow solution, showing a band in red and at least one lipochrome-like band from $\lambda$ 506 to $\lambda$ 483. But the residue was unchanged with iodine in iodide of potassium, became only yellowish with nitric acid and reddish-brown with sulphuric acid. Hence no distinct lipochrome reactions could be obtained. That portion of the residue which was insoluble in alcohol went into water, forming a pale-yellow solution free from bands.

Hence *Leuconia Gossei* contains a trace of chlorophyll, a trace of a histohaematin, and a pigment resembling a lipochrome, but different from one.

*Pachymatisma Johnstonia* (?) This was a sponge whose species was doubtful. It was a dirty yellow colour. A piece of sponge absorbed some of the violet end, and showed a band at blue end of green. Neither in portions of the sponge itself nor in its glycerin extract could I detect a histohaematin band.

The rectified spirit extract was pale-yellow, and showed a faint chlorophyll band in red, and another between green and blue. The former from about $\lambda$ 674·5 to $\lambda$ 656, and the latter about $\lambda$ 502 to $\lambda$ 475. On evaporation a yellow residue was left which was partially soluble in absolute alcohol, the remainder being soluble in water, forming a yellow solution free from bands but absorbing the violet end of spectrum. The filtered absolute alcohol extract of this residue was yellow, and showed the same chlorophyll band in red as before and the same lipochrome-like band. It left on evaporation a yellowish-green residue, which was unchanged by iodine in iodide of potassium, became reddish-brown with sulphuric acid, and a dirty blue which soon passed away with

nitric acid. Hence this sponge contained a trace of chlorophyll and a lipochrome-like pigment.

*Summary and Remarks.* Out of these twelve species of sponge examined by me the following ten contained chlorophyll, namely:—

*Halichondria panicea.*
*Halichondria caruncula.*
*Halichondria rosea.*
*Halina Bucklandi.*
*Halichondria incrustans.*
*Halichondria seriata.*
*Grantia coriacea.*
*Halichondria sanguinea.*
*Leuconia Gossei.*
*Pachymatisma Johnstonia.*

Adding to these the seven, in the solutions of which Krukenberg observed a band in red, we have seventeen sponges containing chlorophyll. And adding *Spongilla fluviatilis,* eighteen. Lipochromes occur in nearly all; and a histohaematin in—

*Halichondria panicea.*
*Halichondria incrustans.*
*Hymeniacidon albescens.*
*Halichondria albescens.*
*Leuconia Gossei.*
*Halichondria rosea.*
*Halina Bucklandi.*

A pigment resembling a "floridine" occurs in *Halichondria rosea,* but with a different spectrum, and owing to dearth of material I was unable to fully examine it.

A "uranidine" occurs in *Grantia coriacea,* and this in aqueous solution gave a band at *D,* sp. 15, which Krukenberg does not mention. With regard to the chlorophyll I have no hesitation in saying that no difference worth mentioning was observed between this and vegetable chlorophyll. The lipochrome constituent (or constituents) reacted differently to the lipochrome of plant chlorophyll, as it remained unchanged with iodine in iodide of potassium, and the fractional method did not separate the chlorophyll constituents so completely as in the case of plant chlorophyll. These results prove that the chlorophyll is of purely animal origin, for similar reactions were obtained by me in the

case of enterochlorophyll[1]. I am using the word chlorophyll now for the mixture of colouring matters, which latter Hansen calls "chlorophyll green" and "chlorophyll yellow," but if one were to restrict the name chlorophyll to the former constituent only, then it could be said that sponge chlorophyll is identical with that of plants. The objection might be made that the fractional method shows a difference; certainly such is the case as far as the colour of the solutions is concerned, and the division of the constituents between the bisulphide and the spirit, but in the case of sponge-chlorophyll we must remember that we have a relatively large amount of lipochromes, and a relatively small amount of "chlorophyll-green," whereas in the case of plants the reverse is the case. But even in the latter the fractional method does not completely separate the green from the yellow constituent, and if relatively more yellow were present, the pure green colour of the bisulphide would not be so well marked as it is.

Another argument in favour of the animal origin of these pigments rests on the fact that if they were due to the presence of unicellular algae living in the sponge tissues we should find these algae microscopically, and detect cholorofucin[2] in the solutions spectroscopically, but such is not the case. Freshly frozen sections failed to reveal the presence of the former even when examined with powers up to 1250 diameters, while an inspection of the accompanying spectrum-maps (Pl. I.) shows that the latter was absent. Marine algae of various kinds are often to be found growing into the sponge substance, but I always took care in cutting up the sponges to select those portions which were free from them.

Hence it may safely be concluded, (1) that chlorophyll is present in sponges, (2) that it is built up synthetically by them. The question now arises of what use is it to sponges? It cannot be of use for mere surface colouration, as its colour is in most cases disguised by yellow, red, or other colour, and it must therefore be of use either for purposes of assimilation, as in plants, or for respiration. In *Halichondria panicea* the chlorophyll is much more apparent in the external parts of the sponge, and it is therefore likely that it has something to do with the absorption of light rays. One cannot help thinking that the very peculiar

[1] *Philos. Trans.* Part I. 1886. I have not adopted the saponification method in this research as Krukenberg has done so already, and he finds that it decomposes the "hepatochromatic colouring stuffs," but is necessary for the complete separation of the lipochromes.

[2] *Quart. Journ. Micros. Soc.* 1887, p. 573—590.

absorption spectrum of chlorophyll indicates a property which is peculiar to it, and which enables it to sift out rays of a certain wave-length to be utilised in the synthesis of the carbohydrates, &c., for although Professor Sachs[1] states that, "on the whole, investigations on the spectrum of chlorophyll have hitherto yielded no facts of any physiological value,—i.e. we should know quite as much of the physiological functions of chlorophyll if its spectrum were absolutely unknown to us," yet Vines[2] has clearly shown that the experiments of Draper, upon which Sachs bases this opinion, were not made with a pure spectrum. And the experiments of Timiriazeff[3] and Engelmann[4] have clearly shown "that the decomposition of carbon dioxide by green plants is most active in those parts of the solar spectrum which correspond to the more conspicuous absorption bands of the chlorophyll spectrum." I may here add that Pringsheim's "screen theory" cannot apply to sponge-chlorophyll for reasons which it is unnecessary to give.

The proof of the co-existence of starch with chlorophyll seems necessary before one can convince botanists that an animal chlorophyll can exist, but this assumption rests on ignorance of well-established botanical facts. In the first place, no starch is produced except the chlorophyll corpuscles are exposed to a bright light, and secondly, in some cases no starch can be found in them; thus Sachs says[5], "In some cases it is impossible directly to observe starch as the product of assimilation in the chlorophyll grains. I found this to be the case in the leaves of our common onion (*Allium cepa*), where, however, large quantities of glucose (sugar) are to be recognised as the result of assimilation....It was observed later, that in the leaves of *Strelitzia* and *Musa* also fatty oils are found as a rule in the chlorophyll instead of starch. It thus appears that in many plants the starch produced in the chlorophyll may be at once transformed into fat, as may also be the case with some species of *Vaucheria*".

In connexion with this observation the fact is striking that in sponges, and indeed in the case of entero-chlorophyll also, the chlorophyll appears to be mostly dissolved in oil, and even in plants Pringsheim's researches (which however Sachs rejects) have shown that "the protoplasmic matrix" (of the chlorophyll grain) "is of spongy texture

[1] *Lectures on the Physiology of Plants*, Eng. trans. p. 322.
[2] Vines. *Lectures on the Physiology of Plants*, p. 156 etc.
[3] *Ann. de Chim. et de Phys.* Sér. 5, XII. 1877.
[4] *Bot. Zeitg.* 1882 and 1883.
[5] *Lectures on the Physiology of Plants*, p. 310 seq.

and forms a toughish framework, honeycombed by innumerable minute cavities. The interstices of this framework are everywhere permeated by an oily fluid called *hypochlorin*, which may be extracted by proper treatment, and is found to be of a somewhat crystalline nature. The green colouring matter is held in solution in this oil.[1]" Then, again, Godlewski showed in 1873 that in an atmosphere devoid of $CO_2$ no starch is produced in the chlorophyll corpuscles, even in the light[2], and that very large quantities of carbon dioxide in the air prevent the formation of starch.

The fact, that in sponges the chlorophyll is accompanied or often replaced by the lipochromes, would go to show that the step from a lipochrome to a chlorophyll is not a great one, and it is highly probable that these colouring matters are concerned in the formation of fatty matters, perhaps from the waste carbon dioxide given off during the metabolic changes of the tissue products, and from the water in which they are bathed.

I advanced the theory which Regnard's researches[3] seemed to suggest—that chlorophyll may be really a respiratory pigment—in a paper on the Chromatology of *Anthea cereus*[4], also in another mentioned in the foot-note; and more recently Dr. Schunck[5] has stated his belief that in plants it is concerned in respiration, but he suggests that it may be a carbonic-acid carrier, not an oxygen carrier. This theory would, if it turned out to be correct, explain the function of animal chlorophyll, but that it takes up $CO_2$ merely for the purpose of giving it up again to the surrounding protoplasm is a doubtful advantage to the animal. If on the other hand it could remove the waste $CO_2$, and then by the influence of light rays build up from $CO_2$ and water, some substances, such as starch, glycogen, sugar or fat, which are of direct service to the animal, it would be of great use in the constructive metabolism of animals. And this is most probably the function which it does perform, judging from all recent botanical knowledge[6]. Possibly, however, it may be a bye-product, but this is unlikely.

[1] Behrens. *Text-book of General Botany*, Eng. ed. p. 262.

[2] Sachs. *loc. cit.*

[3] *Compt. rend.*, CI, 1293—1295, and *Journ. Chem. Soc.* March 1886, p. 254.

[4] *Quart. Journ. Micros. Soc.*, *loc. cit.*, and *Proc. Birm. Philos. Soc.* Vol. v. part I. p. 212.

[5] Opening Address Sect. B. Brit. Assoc. Meeting, 1887.

[6] In the case of enterochlorophyll it is possible that we have to do with a body which is either concerned in the synthesis of the carbohydrates, or is itself a bye-product of the synthetic process (constructive metabolism), which is now known to take place in the so-called "liver" of Invertebrates. The term "digestive gland" used by Howes and Scott in the extended edition of Huxley's Biology is more appropriate than "liver."

The extraordinary richness of sponges in fatty and ethereal oils, taken in connexion with the peculiar ozone-like smell which they give off, led Krukenberg to search for ozone, but without result.

Finally it may be remarked, that the union between oxygen and chlorophyll cannot be a loose combination, as it is in the case of haemoglobin, the oxygen must enter into an intra-molecular combination with the chlorophyll if at all, as the action of reducing agents teaches this fact. Besides we know that all respiratory pigments, except a few, such as echinochrome and aphidein, are united to proteids, and when separated from them are no longer respiratory; and the observations which I have made show that a histohaematin which is of respiratory use may coexist with chlorophyll in sponges; this would be a superfluous advantage if both pigments were respiratory in the same sense. It is possible that they may supplement each other, the histohaematin uniting with oxygen and the chlorophyll with the carbon dioxide.

In the accompanying chart of spectra it will be seen that in many cases the whole series of chlorophyll bands are shown. In Krukenberg's drawings, as a rule, only the dominant band. This doubtless was due to the fact that he worked with dilute solutions or examined layers of fluid too thin to show all the bands.

Owing to the difficulty of reading the lipochrome bands, I am not by any means certain that the measurements given here are accurate, and they must not be accepted as final. In many cases it is impossible to say where the shading begins or ends.

## EXPLANATION OF CHART OF SPECTRA. Pl. I.

Sp. 1. Absolute alcohol extract of *Halichondria panicea.*

2. After diluting with water this was agitated with bisulphide of carbon, which showed this spectrum in a deep layer.

3. The same thinner layer showing the lipochrome bands.

4. Glycerin extract of *Halichondria panicea*, showing bands of a histohaematin.

5. Alcohol extract of *Halichondria caruncula.*

6. Alcohol extract of a residue left by evaporating an alcohol solution of the chlorophyll of same sponge.

7. Aqueous solution of *Halichondria rosea.*

8. Rectified spirit extract of same sponge.

9. The same thinner layer: to show the lipochrome bands.

10. Lipochrome bands in bisulphide of carbon from same sponge (see description in paper).

Absolute alcohol extract of *Halina Bucklandi.*

12. The same thinner layer.

13. Histohaematin band in sponge itself: *Halichondria incrustans.*

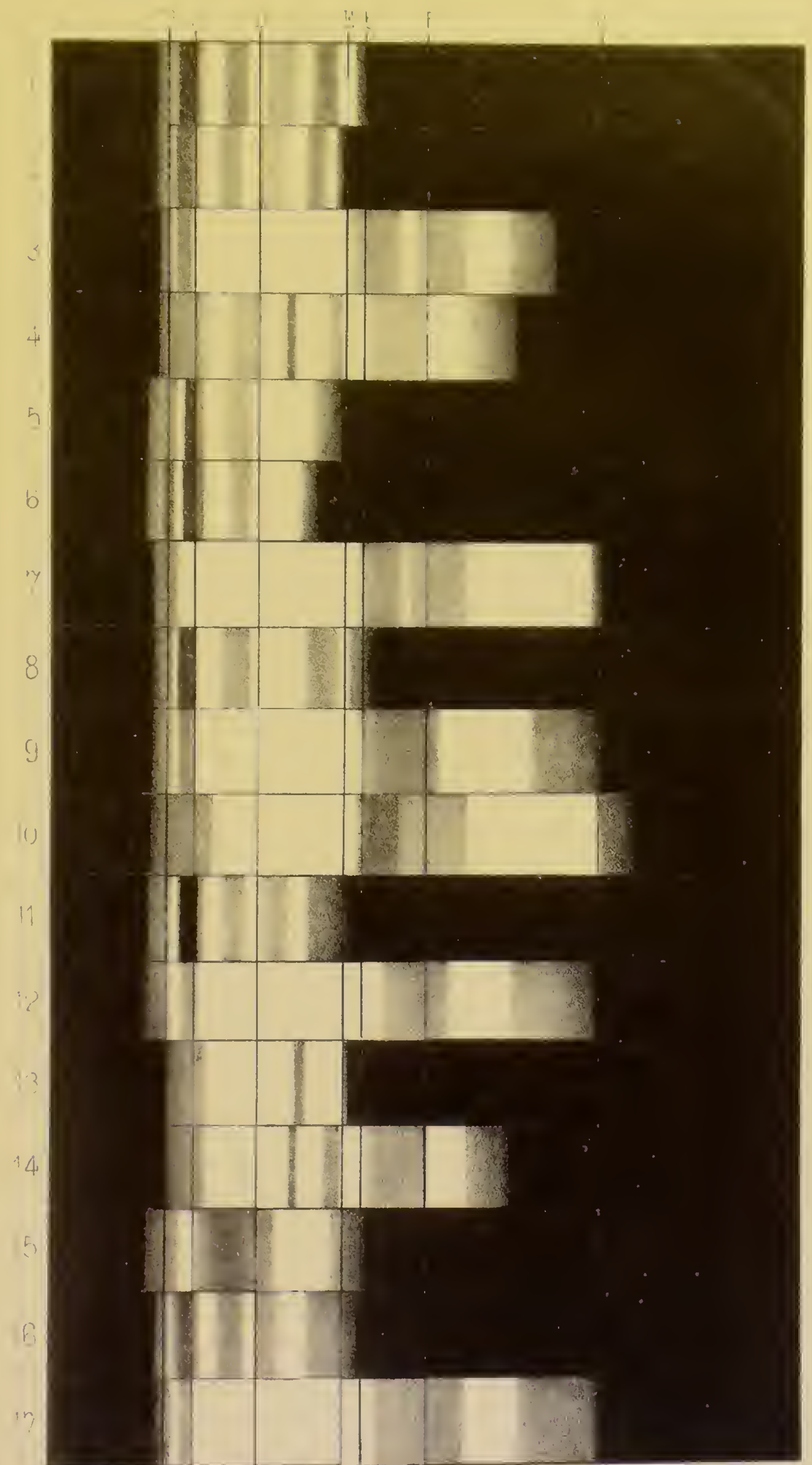
14. Glycerin extract of same sponge showing the histohaematin bands.

15. The blackish-green uranidine of *Grantia coriacea* in water. (See description in paper.)

16. Rectified spirit extract of *Halichondria sanguinea.*

17. The same thin layer.

All these were mapped from the microspectroscope and cannot be relied upon for wave-length measurements, the latter were calculated from a chemical spectroscope of greater dispersion.

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